PB84-130392

Diets for 'Ceriodaphnia reticulata' Life Cycle Tests

(U.S.) Environmental Research Lab.-Duluth, MN

1983

U.S. Department of Commerce
National Technical Information Service
NTIS

PB84-130392

EPA-600/D-84-021
1983

DIETS FOR CERIODAPHNIA RETICULATA LIFE CYCLE TESTS

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

Teresa J. Norberg and Donald I. Mount
Environmental Research Laboratory-Duluth
U.S. Environmental Protection Agency
6201 Congdon Boulevard
Duluth, Minnesota 55804

ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
DULUTH, MN 55804

TECHNICAL REPORT DATA (Please read Instructions on the reverse before completing)		
1. REPORT NO. EPA-600/D-84-021	2.	3. RECIPIENT'S ACCESSION NO. PB84 130392
4. TITLE AND SUBTITLE Diets for Ceriodaphnia reticulata Life-Cycle Tests		5. REPORT DATE 1983 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) T.J. Norberg and D.I. Mount		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Environmental Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Duluth, MN 55804		10. PROGRAM ELEMENT NO. 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Same as above		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA-600/03

15. SUPPLEMENTARY NOTES

ASTM Aquatic Toxicology Symposium Series

16. ABSTRACT

Two diets were compared for the cladoceran, Ceriodaphnia reticulata for use in culturing and life-cycle toxicity tests. One diet is a suspension of dry yeast dissolved in distilled water and fed at a rate of 250 µg per animal. The other diet is prepared from frozen adult brine shrimp and distilled water. The aged supernatant is fed using 0.05 mls per animal. Animals were fed daily and water was changed three times each week. The feeding study was conducted for ten generations using young per female and adult survival as endpoints. The young production per female of surviving adults is comparable but the longevity of the animals fed the yeast diet was considerably greater than the animals fed the shrimp diet.

17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) unclassified 20. SECURITY CLASS (This page) unclassified	21. NO. OF PAGES 25 22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

ABSTRACT

Two diets were compared for the cladoceran, Ceriodaphnia reticulata for use in culturing and life-cycle toxicity tests. One diet is a suspension of dry yeast dissolved in distilled water and fed at a rate of 250 ug per animal. The other diet is prepared from frozen adult brine shrimp and distilled water. The aged supernatant is fed using 0.05 mls per animal. Animals were fed daily and water was changed three times each week. The feeding study was conducted for ten generations using young per female and adult survival as endpoints. The young production per female of surviving adults is comparable but the longevity of the animals fed the yeast diet was considerable greater than the animals fed the shrimp diet.

KEY WORDS: Ceriodaphnia reticulata, cladocerans, chronic bioassay, food quality, culture methods, aquatic toxicology

Introduction

Ceriodaphnia reticulata recently has been recommended as a test species for acute and chronic toxicity testing (1)[1]. The growth, survival, and reproduction of cladocerans in such tests is highly dependent on the condition of the animals which in turn is dependent in part on the quality and quantity of the food. The rationale for testing cladocerans is outlined in the ASTM Proposed Standard Practice for Conducting Renewal Life-cycle Toxicity Tests on *Daphnia magna* (Draft No. 1) being developed by ASTM Committee E-47 on Biological Effects and Environmental Fate. The same rationale is applicable to *Ceriodaphnia reticulata*.

Ceriodaphnia are common with world-wide distribution and can be found in both limnetic and littoral zones. *C. reticulata* and other species of *Ceriodaphnia* are the preferred food for fish larvae and are of great importance as food for the early stages of fry development of various species of fish (2,3). While the preferred food type for *Daphnia magna* has been examined extensively (4,5,6,7, ASTM Draft No. 1) only limited examination of the preferred food of *Ceriodaphnia* has been done (8,9). One such experiment (9) found *C. reticulata* to assimmilate much less algae when fed a mixed diet of algae and bacteria than when fed algae alone. No change in feeding on bacteria was seen when algae was added indicating the preferential assimilation of bacteria by *Ceriodaphnia* and ingestion occurred rapidly, taking less than five minutes. Another study (8) of the preferred food type was done with *C. quadrangula* fed

two diets. One an algal mixture consisting mainly of Scenedesmus quadricauda and the other diet was an "Artemia water" which was filtered pond water in which naupliar larvae of Artemia salina had died and begun to decompose. The Ceriodaphnia population in the "Artemia water" grew much better due to the high number of microorganisms available in the water. The "Artemia water" had a bacterial count of about 10×10^{12} bacteria/ml while the algal culture bacterial count was 10×10^{6} bacteria/ml. Further study by these authors found microbial populations actually increased feeding and population size of Ceriodaphnia while a bacteria-free Scenedesmus culture resulted in a rapid decline in the population over a 34 day period. A recent study (10) found the species composition of filter feeding zooplankton is strongly influenced by the amount of suspended bacteria available and bacteria were determined to be an important food source for filter feeding cladocerans with fine mesh sizes of filtering apparatus, for species such as Ceriodaphnia quadrangula and Daphnia magna. A number of authors (8,9,10, 11) have noted that Ceriodaphnia and Daphnia magna feed on bacteria as efficiently as unicellular algae.

Ceriodaphnia have been maintained continuously at the Environmental Research Laboratory-Duluth (ERL-D) in a flow-through system and feed on bacteria using trout food as the bacterial food source. Preliminary studies indicated the two diets discussed in this paper, a yeast diet and a shrimp diet, to be adequate for reproduction and growth. The objectives of this paper are to present the results of a ten generation feeding study to test the suitability of the two diets for culturing

Ceriodaphnia and for use in life-cycle renewal toxicity testing. Recommendations on food type, concentrations of food for culturing and toxicity tests, and guidance on the acceptable number of young for a seven day life-cycle test will also be presented.

Methods

The Ceriodaphnia reticulata used in the feeding study had been in continuous culture at the ERL-D for three years. Two diets were fed for ten generations and the number of young per female were counted. The F_0 generation animals were collected from five adults and the young to begin the study were less than two hours old. Ten animals per diet were used for each generation. Young production and adult survival were monitored for 28 days for each generation. Successive generations were begun with less than 24 hour old young produced when adults of each generation were 14 days old. In two generations of animals fed the yeast diet and three generations of animals fed the shrimp diet, the successive generation was started before the parental generation reached 14 days. The young to begin the next generation were collected from the maximum number of surviving females. One Ceriodaphnia per 30 ml beaker with 15 ml of Lake Superior water was used. The dilution water was changed three times a week and any young were counted and discarded.

The two diets were prepared as follows. The yeast diet was a suspension of dry bakers yeast dissolved in distilled water and fed at a rate of 250 ug per animal. A 5000 mg/l yeast stock was prepared weekly

and 0.05 mls per 15 mls of water was fed daily. The concentration of the yeast in 15 mls was 16.7 mg/l. The yeast solution was shaken vigorously prior to use and new stock solution was prepared every week.

The other diet, a shrimp diet, was initially prepared using 10 grams of frozen adult brine shrimp mixed with 100 mls of distilled water, stirred for three hours and refrigerated. Thereafter each week 50 mls of the clear supernatant was removed and added to 50 mls of distilled water and 10 grams of frozen brine shrimp. The shrimp food was shaken vigorously after feeding. The shrimp diet was fed at a rate of 0.05 mls clear supernatant per 15 mls of water.

Dissolved oxygen levels of the test water remained above 5 mg/l over the study period. The pH of the water was 7.9 and hardness was 48 mg/l $CaCO_3$.

Results

A ten-generation feeding study with Ceriodaphnia reticulata was run to evaluate the suitability of two diets for culturing and life-cycle renewal toxicity testing. Young production per female fed each diet for ten generations was determined. The number of young, the adequacy of each diet, and survival of the adults are discussed.

The cumulative mean number of young per surviving female for the first 21 days of the 28 day study for each generation fed the yeast diet are shown on Figures 1, 2, and 3. Survival was recorded and young counted three times a week. ANOVA and Dunnett's test were run on the mean number of young per female reproductive day for the entire 28 day

study using the F_0 generation as the control treatment. Total young produced by each female was divided by her reproductive days to obtain the mean number of young per female per day. Since C. reticulata produce their first brood between 72 and 96 hours at 25 C, three days were subtracted from the total days each animal lived to determine the number of reproductive days. The mean number of young for generations F_1, F_2, F_5, and F_6 were significantly different ($p = 0.95$) from the F_0 generation. The F_1 generation had a higher mean number of young than the F_0 generation. Two females in the F_2 died at seven days prior to producing any young, which caused the lower value for the F_2 generation. Poor water quality of the dilution water was responsible for low young production for the F_5 and F_6 generations.[1]

ANOVA and Dunnett's test were run on the young production for 7, 14, and 21 days for animals fed the yeast diet. The 7 day interval has four reproductive days and the mean number of young per day per female for the F_1 and F_3 generations were significantly higher ($p = 0.95$) than the F_0 generation. The F_0 generation had a mean number of young per reproductive day of 2.6 while the F_1 and F_3 generations produced 4.0 and 4.5 mean young per day respectively. The analysis at the 14 day interval or 11 reproductive days resulted in additional generations showing significantly more young per day. The water quality problems in the F_6 generation caused the young production to be significantly lower than the value for the F_0 generation. At 21 days, the F_1 generation was significantly greater ($p = 0.99$) and none of the F_6 generation survived to 21 days. The 7, 14 and 21 day values included only the data from

[1] The water supply from Lake Superior infrequently causes problems in the cladoceran culture in the spring and fall. Further details are in the Discussion section.

adults that lived to each time interval rather than comparing the mean number of young per reproductive day for all animals as was done in the 28 day evaluation.

The cumulative mean number of young per female fed the shrimp diet is similar to that of animals fed the yeast diet (Figures 3, 4 and 5) for the first 21 days of the 28 day feeding study. The 21 day period is shown since survival to 28 days with adults fed the shrimp diet occurred only during one generation. ANOVA and Dunnett's test on the mean number of young per female per reproductive day for the 28 day study found no generation to be significantly different from the F_0 generation. ANOVA and Dunnett's test were run at the 7 and 14 day intervals on the mean number of young per female using the F_0 generation as a control treatment. The 7 day period (4 reproductive days) for the F_9 generation was significantly greater than ($p = 0.95$) the F_0 generation. The mean number of young was 1.7 and 3.0 per reproductive day for the F_0 and F_9 generations, respectively. The analysis at 14 days resulted in the F_4 and F_9 generations being significantly higher ($p = 0.95$) than the F_0 generation. The 21 day comparison of the mean number of young could not be done using the F_0 generation as the control treatment due to only 10% survival of the original adults at 21 days.

Survival of adults fed the shrimp diet was less than the yeast diet. Time to 50% survival for the adults of each generation for the 28 day study was less with each generation fed the shrimp diet compared to animals fed the yeast diet (Table 1). The mean survival of ten generations

on the yeast diet was 54.6% at 21 days and 39.6% at 28 days. For ten generations on the shrimp diet, mean survival of the adult Ceriodaphnia at 21 days was 36.1% and at 28 days was 21%.

Data on the cumulative number of young from all the Ceriodaphnia from all generations fed the yeast diet which lived to 28 days was averaged. The mean number of young per female was 14.2 at 7 days, 58.8 at 14 days, 90.8 at 21 days and 123.4 young at 28 days. Because survival was better for adults fed the yeast diet, regression of the cumulative mean number of young for 21 days of each generation were run. Figure 7 is the plot of the birth rate regression slope versus generation number for animals fed the yeast diet to obtain the birth rate during the study. The graph indicates the rate of birth to be similar for most generations with the exceptions of generations F_1, F_6, and F_7. Both the F_1 and F_7 generations produced more young than the other generations while the F_6 generation produced less young because of the water quality problems. The plot clearly shows no upward or downward trend in the birth rate during the ten generation study.

Discussion

Survival and reproduction are often the determinants for the effect concentration in life-cycle toxicity tests. ASTM suggests that as a minimum an average of 40 young per Daphnia magna be produced in 21 days for an acceptable life-cycle test. Similar guidance is needed for

C. reticulata testing. As the life-cycle C. reticulata test method (1) recommends a seven day test period, it seems reasonable that the minimum acceptable number of young produced in a life-cycle test should be based on the same period. Since there are four reproductive days during a seven day test, the mean number of young per day is 2.96, based on the feeding study. Since some animals may not release their third brood by the end of the test (an average of 2.5 broods per control animal is considered acceptable (1)) the minimum average number of young per animal should be no less than nine. Nine young in seven days is almost identical to the mean number of young (14.7) per female for animals that lived to 28 days and fed the yeast diet minus one standard deviation. It should be noted that this value is for sterile type waters where animals feed primarily on the food provided by the yeast and the minimum number of young in seven days should be exceeded in waters containing additional food.

Information in the literature on C. reticulata (3) fed four species of algae gives some data about their food preference, young production, and average life-span. When fed Chlamydomonas nivalis the average life-span was 17 days with an average number of young of 10. The average life-span when fed Chlorella vulgaris was 20 days with a mean number of young of 13. Twenty-one days was the average life-span when Chlorella elliposoidea was fed and the mean number of young was 18. Animals fed Ankistrodesmus falcatus var. duplex had an average life-span of 22 days and an average of 32 young. Also, the time to reproduction was four days for all but Ankistrodesmus where it was three days. The results

for the time to reproduction with Ankistrodesmus compares with what we have experienced (1) and with other work (11). Another study (13) determined the tropical Ceriodaphnia cornuta produces an average of 123.6 eggs and has a mean life-span of 21.2 days. The number of eggs produced by C. cornuta is very similar to the number of young produced by C. reticulata. But the results of C. reticulata fed algae (3) contrasts sharply to the young production for the yeast and shrimp diets used in this feeding study. For comparison, at 21 days the mean number of young produced for eight generations (F_5 and F_6 excluded for water quality interference) was 88 for animals fed the yeast diet and 95 for animals fed the shrimp diet which is almost three times greater than animals fed algae. The results of the yeast and shrimp diets along with the results of others (8,10,13) strongly support the hypothesis that Ceriodaphnia are primarily bacteria feeders. Survival of adults and time to first brood were generally acceptable for the animals fed the yeast diet. While time to first brood for animals fed the shrimp diet was satisfactory the survival of the adults was considerably lower than yeast fed adults. Because of the ease of preparing and using the yeast diet as well as the better survival and high number of young per female over time, the yeast diet is a better diet than either the shrimp diet or any algae diets described in the literature. Waters containing high populations of microorganisms, such as some river waters, will produce even higher numbers of young (1) which can be attributed to the additional bacteria in the water. The amount of yeast fed the Ceriodaphnia, 250 ug per animal, appears to provide an adequate level of available food while not increasing the suspended solids beyond the recommended levels.

The poor survival of the F_5 and F_6 generations due to water quality problems was unfortunate. As noted previously, problems with the Lake Superior water have been noted with the cladoceran populations at ERL-D in the spring and the fall. The fathead minnow (Pimephales promelas) culture unit using the same water supply experienced limited spawning and reduced fertility at the same time water quality problems were experienced in this feeding study. Other investigators noted mortality of fathead minnows in the early life stage tests during the same period.

The birth rate of Ceriodaphnia for 7 out of 10 generations was very similar. Only the F_6 generation birth rate, occurring during the water quality problem period was lower and the F_1 and F_7 generations had birth rates higher than the other generations. Since the F_7 generation young were taken from the F_6 generation adults and produced more young than the average number of young substantiates that the low production of the F_6 generation was not nutritionally induced. Because survival was erratic in the shrimp diet, the birth rate could not be evaluated.

During the preliminary testing on the food types for C. reticulata the PR-11 and bakers yeast mixture (5) was fed at the rate of 0.1 ml/day to 15 mls of water and one animal. The Ceriodaphnia remained very small, very whitish, and only produced a mean of 12 young per female in 14 days. The Ceriodaphnia fed the yeast or shrimp diet were brownish in coloration and robust in form. The results with Ceriodaphnia fed the

PR-11 diet correlates with the results of the PR-11 fed Daphnia magna (4) which hypothesizes the success of the PR-11 diet to be due to a secondary source of nutrition in the form of bacteria and protozoa.

Conclusions

Comparison of the two diets for 28 days and ten generations strongly suggests the yeast diet is a better choice than the shrimp diet. The 250 µg of yeast per animal appears to provide an adequate diet and can be used with confidence for culturing and life-cycle toxicity tests.

Table 1. Time (days) to 50% Survival for Ceriodaphnia reticulata fed the yeast diet and the shrimp diet for 28 days of the feeding study.

Generation	Yeast diet	Shrimp diet
F_0	21	12
F_1	>28	12
F_2	>28	28
F_3	14	21
F_4	>28	7
F_5	7	5
F_6	10	5
F_7	19	12
F_8	17	10
F_9	21	7

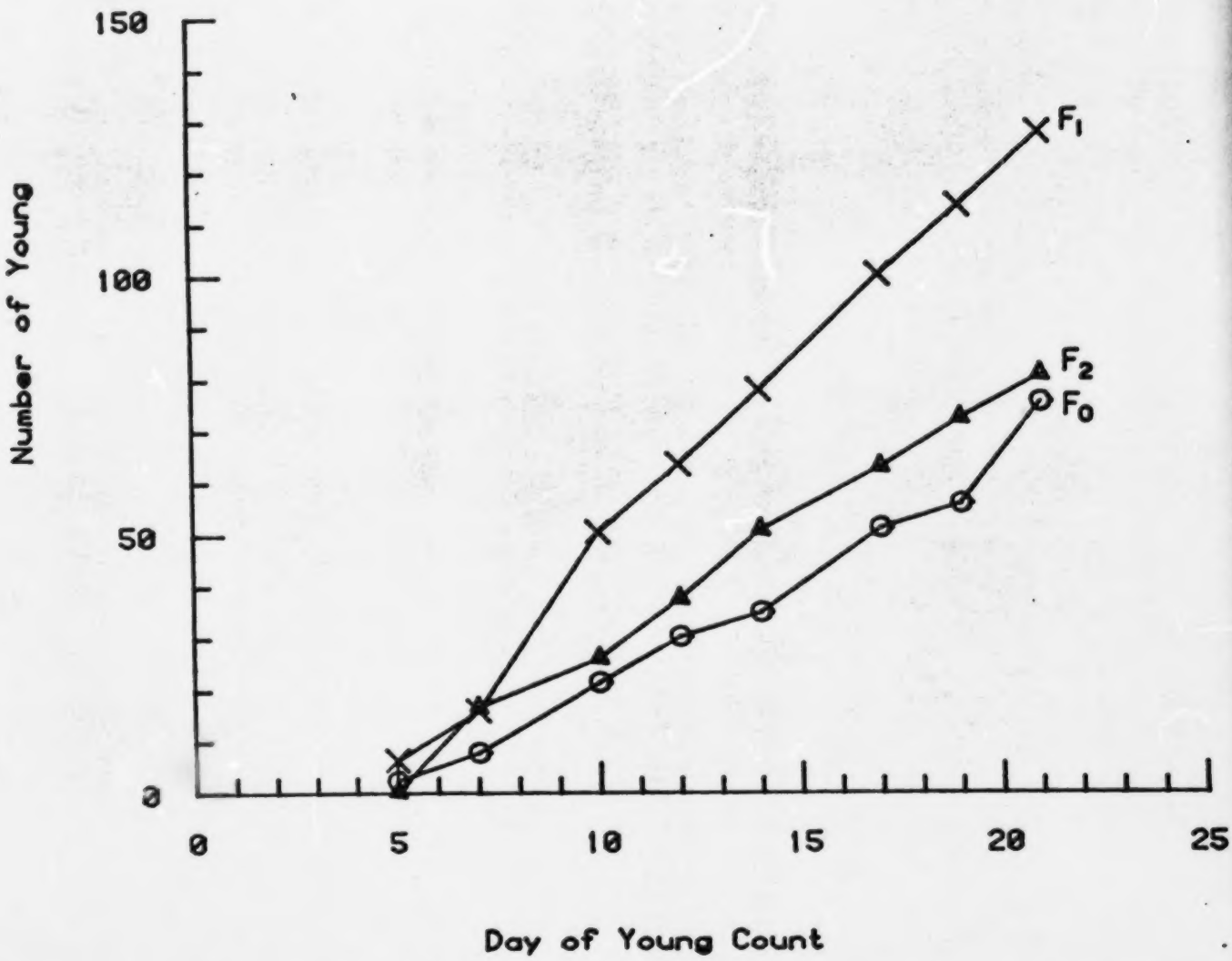

Figure 1. Yeast diet cumulative mean number of young per surviving Ceriodaphnia reticulata for generations F_0, F_1, and F_2. Plot is for the first 21 days of 28 day feeding study.

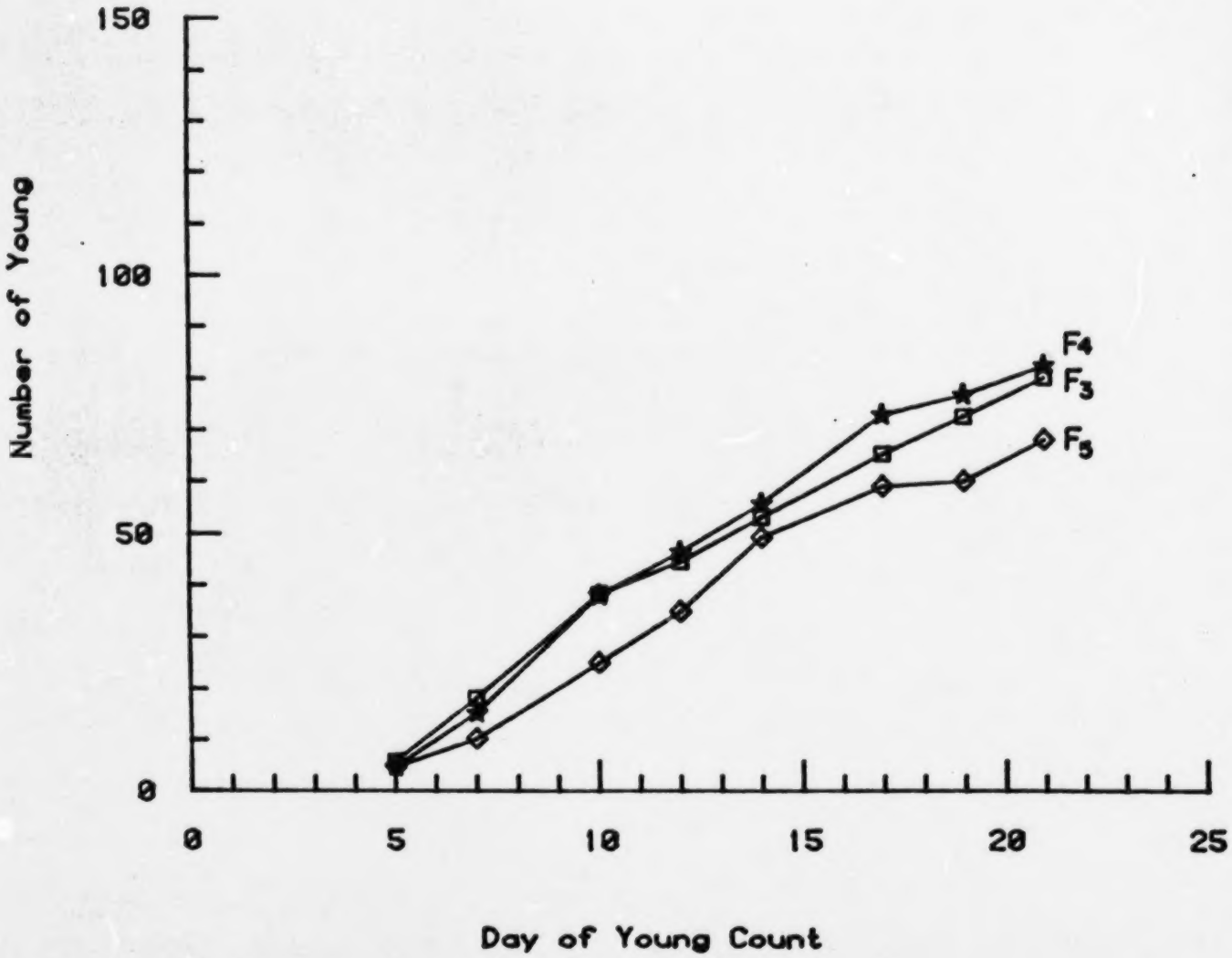

Figure 2. Yeast diet cumulative mean number of young per surviving Ceriodaphnia reticulata for generations F_3, F_4, and F_5. Plot is for the first 21 days of 28 day feeding study.

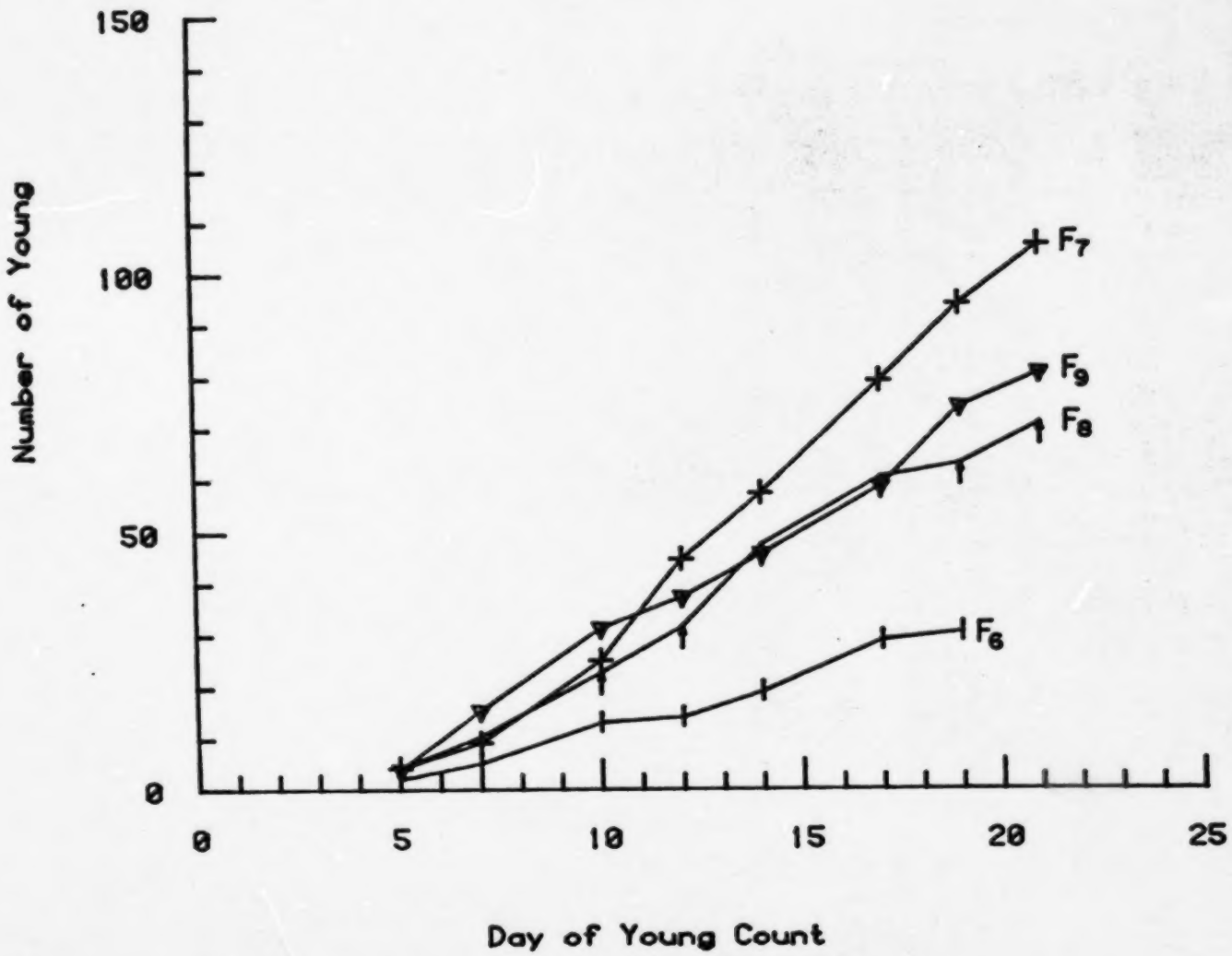

Figure 3. Yeast diet cumulative mean number of young per surviving Ceriodaphnia reticulata for generations F_6, F_7, F_8, and F_9. Plot is for the first 21 days of 28 day feeding study.

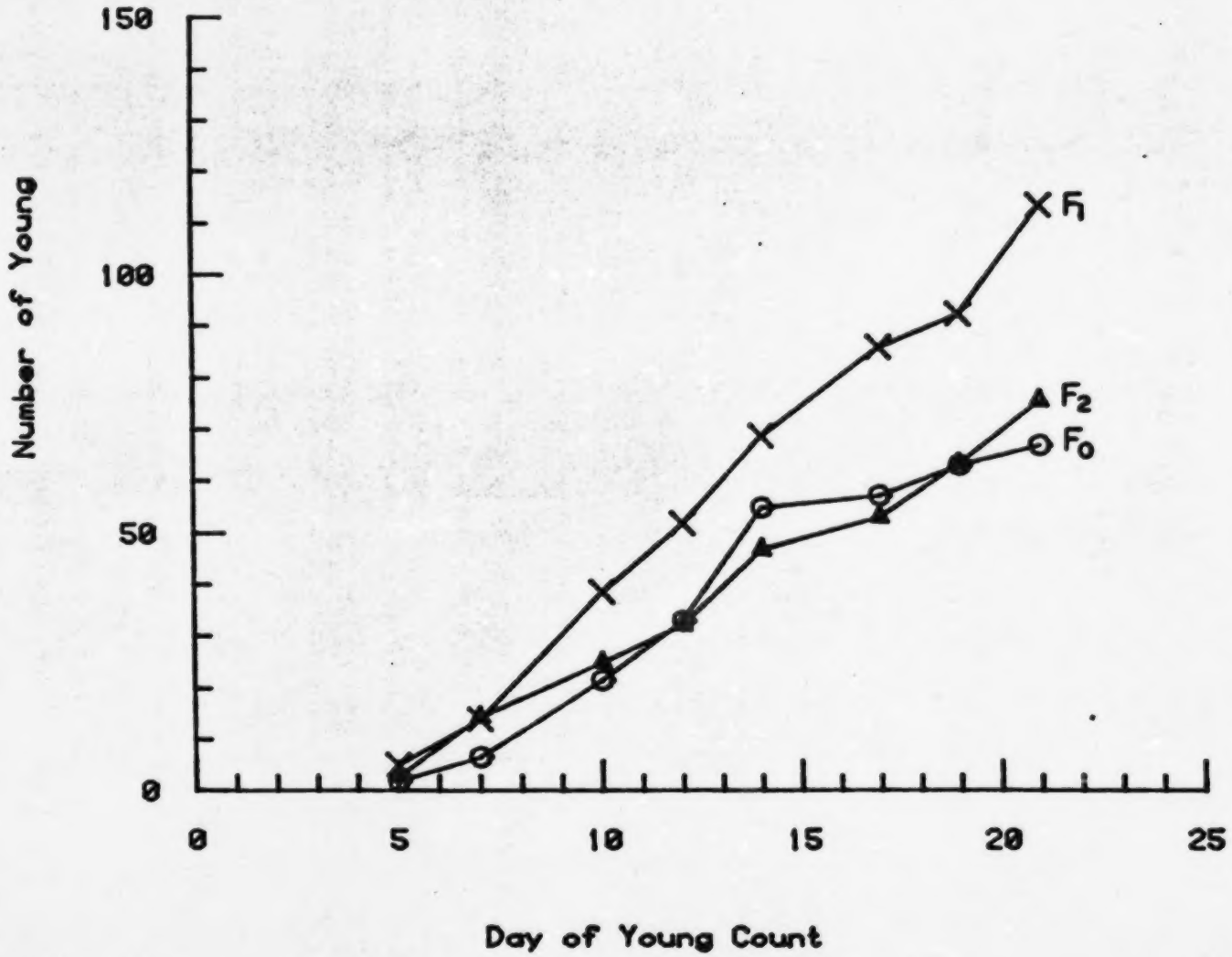

Figure 4. Shrimp diet cumulative mean number of young per surviving Ceriodaphnia reticulata for generations F_0, F_1, and F_2. Plot is for the first 21 days of 28 day feeding study.

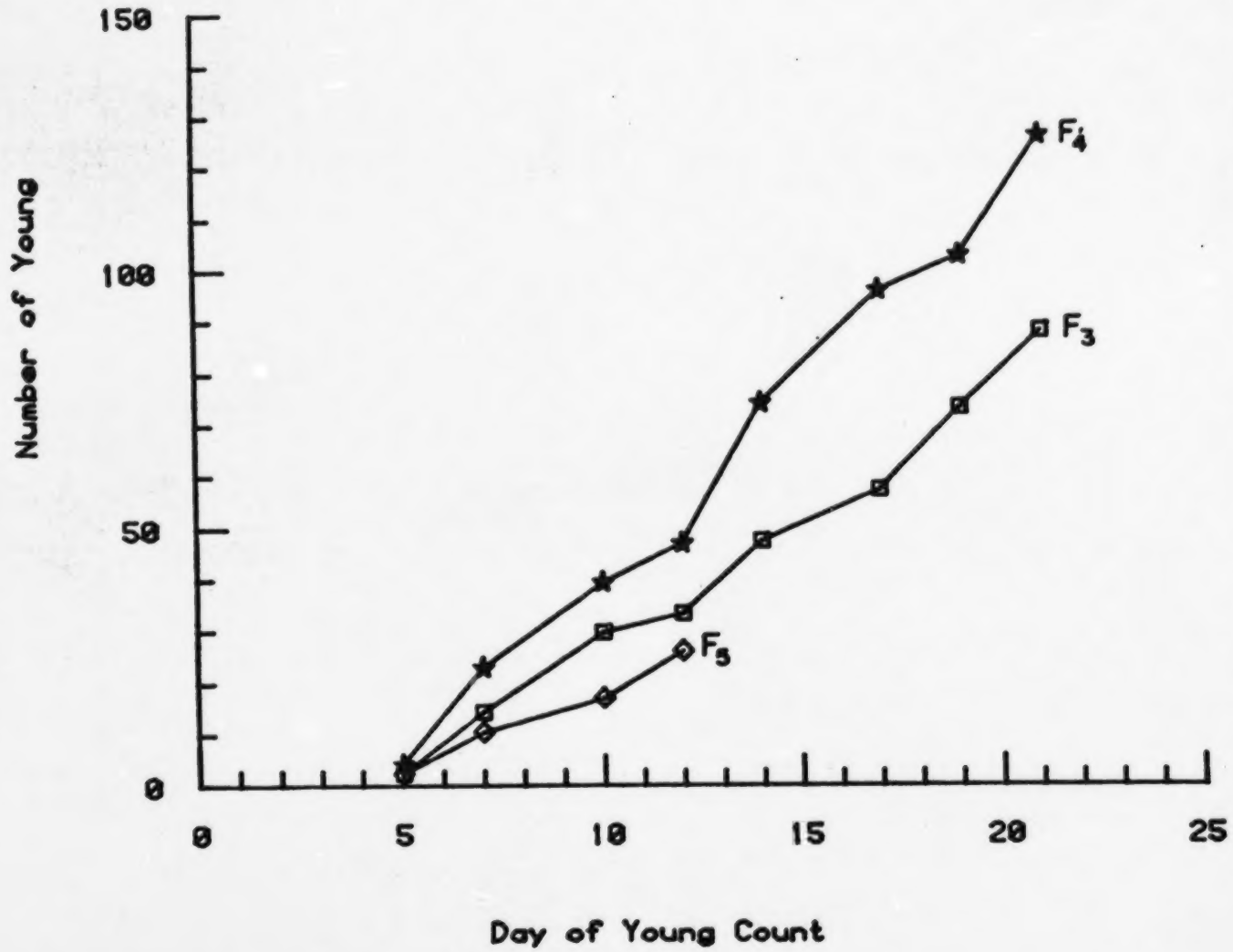

Figure 5. Shrimp diet cumulative mean number of young per surviving Ceriodaphnia reticulata for generations F_3, F_4, and F_5. Plot is for the first 21 days of 28 day feeding study.

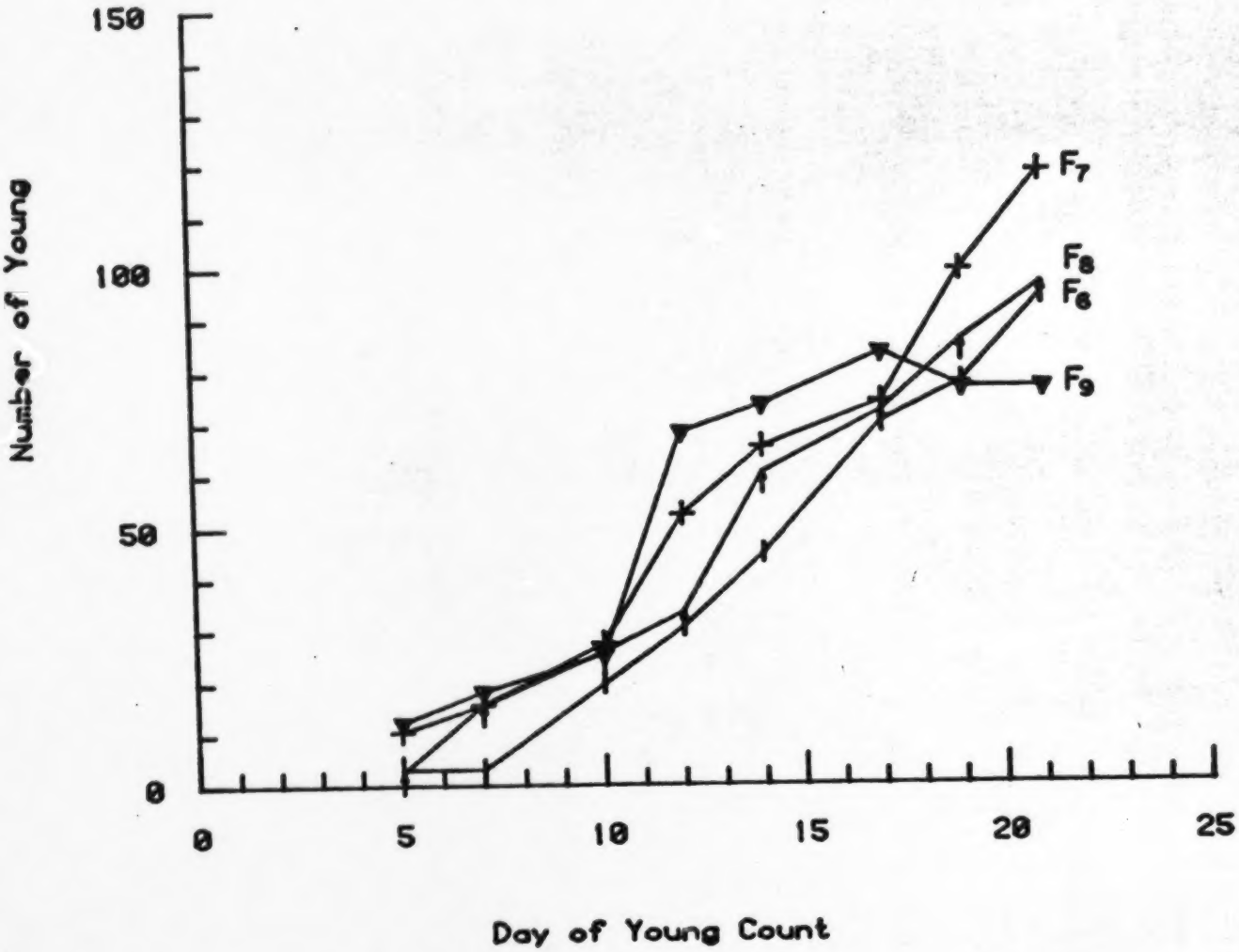

Figure 6. Shrimp diet cumulative mean number of young per surviving Ceriodaphnia reticulata for generations F_6, F_7, F_8, and F_9. Plot is for the first 21 days of 28 day feeding study.

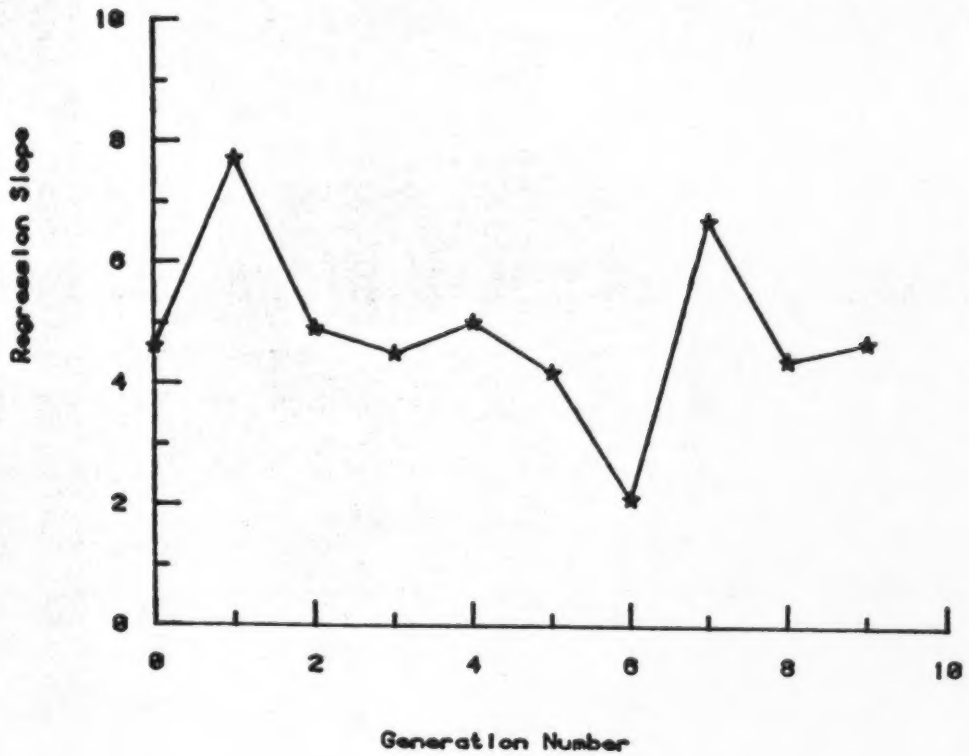

Figure 7. Plot of the birth rate regression slopes versus generation number for Ceriodaphnia reticulata fed the yeast diet (regression is for first 21 days of the feeding study).

REFERENCES

1. Mount, D.I., and Norberg, T.J., A Seven-Day Life-Cycle Cladoceran Test, Manuscript, Environmental Research Laboratory-Duluth, Minnesota.

2. Filatov, V.I., Journal of Icthyology, Vol. 12, No.2, 1972, pp. 812-818.

3. Czeczuga, B., and Bobiatynska-Ksok, E., The Extent of Consumption of the Energy Contained in the Food Suspension by Ceriodaphnia reticulata (Jurine), Proceedings of IBP-UNESCO Symposium on Productivity Problems of Freshwaters, Kazimierz Dolny, Poland, May 6-12, 1970, Z. Kajak and A. Hillbricht-Iikowska, Eds, pp. 739-748.

4. Goulden, C.E., Comotto, R.M., Hendrickson, J.A., Jr., Horning, L.L., and Johnson, K.L., Aquatic Toxicology and Hazard Assessment: Fifth Conference, ASTM STP 766, J.G. Pearson, R.B. Foster, and W.E. Bishop, Eds, American Society for Testing and Materials, 1982, pp. 139-160.

5. Biesinger, K.E. and Christenson, G.M., Journal of Fisheries Research Board of Canada, Vol. 29, 1972, pp. 1691-1700.

6. Hutchinson, G.E., A Treatise on Limnology, Vol. II, New York, 1967.

7. Banta, A.M., Studies on the Physiology, Genetics and Evolution of Some Cladocera, Carnegie Institution of Washington, Publication No. 513, 1939, pp. 1-285.

8. Smyly, W.J.P. and Collins, V.P., Freshwater Biologicia, Vol. 5, 1975, pp. 251-256.

9. Gophen, M., Cavari, B.Z., and Berman, T., Nature, Vol. 247, 1974, pp. 393-394.

10. Geller, W. and Muller, H., The Filtration Apparatus of Cladocera: Filter Mesh-Sizes and Their Implications on Food Selectivity, Oecologia (Berlin, Vol. 49, 1982, pp. 316-321.

11. Askenova, Ye.I., Bogucharskova, G.I., and Zozulina, M.I., Hydrobiologica Journal, Vol. 5, No. 5, 1969, pp. 19-26.

12. Shuba, T. and Costa, R.R., Transactions of the American Microscopy Society, Vol. 91, No. 3, 1972, pp. 429-435.

13. Murugan, N., Journal of Inland Fisheries Society of India, Vol. 7, 1975, pp. 80-87.